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Journal
Journal of Mammalogy, 100(5)

ISSN
0022-2372

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Publication Date
2019-10-22

DOI
10.1093/jmammal/gyz128

Peer reviewed

Journal of Mammalogy, 100(5):1546–1563, 2019
DOI:10.1093/jmammal/gyz128

Geographic variation and evolutionary history of *Dipodomys nitratoides* (Rodentia: Heteromyidae), a species in severe decline

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We examined geographic patterns of diversification in the highly impacted San Joaquin kangaroo rat, *Dipodomys nitratoides*, throughout its range in the San Joaquin Valley and adjacent basins in central California. The currently recognized subspecies were distinct by the original set of mensural and color variables used in their formal diagnoses, although the Fresno kangaroo rat (*D. n. exilis*) is the most strongly differentiated with sharp steps in character clines relative to the adjacent Tipton (*D. n. nitratoides*) and short-nosed (*D. n. brevinasus*) races. The latter two grade more smoothly into one another but still exhibit independent, and different, character clines within themselves. At the molecular level, as delineated by mtDNA cytochrome *b* sequences, most population samples retain high levels of diversity despite significant retraction in the species range and severe fragmentation of local populations in recent decades due primarily to landscape conversion for agriculture and secondarily to increased urbanization. Haplotype apportionment bears no relationship to morphologically defined subspecies boundaries. Rather, a haplotype network is shallow, most haplotypes are single-step variants, and the time to coalescence is substantially more recent than the time of species split between *D. nitratoides* and its sister taxon, *D. merriami*. The biogeographic history of the species within the San Joaquin Valley appears tied to mid-late Pleistocene expansion following significant drying of the valley resulting from the rain shadow produced by uplift of the Central Coastal Ranges.

Key words: colorimetrics, *Dipodomys nitratoides*, morphometrics, mtDNA, phylogeography, San Joaquin kangaroo rat, systematics

The San Joaquin kangaroo rat (*Dipodomys nitratoides*) has one of the smallest geographic ranges of any species in the genus, limited to the southern half of the San Joaquin Valley in central California, which, in turn, is one of the most intensively modified landscapes within the United States. Currently three subspecies are recognized (Best 1991; Williams et al. 1993), two of which (the Fresno kangaroo rat, *D. n. exilis*, and the Tipton kangaroo rat, *D. n. nitratoides*) are listed as Endangered under the Federal Endangered Species Act and the third (the short-nosed kangaroo rat, *D. n. brevinasus*) is considered a California Species of Special Concern by the California Department of Fish and Wildlife. Although to different extents, all three taxa have suffered range retraction as the completion of major water distribution projects post-World War II resulted in rapid conversion of the native saltbush scrub, alkali sink, and grassland communities to agriculture (Preston 1981; Kelly et al. 2005; see also Supplementary Data SD1, which both provides comparison maps of historical [pre-European] habitats and contemporary land use but also identifies place names for readers who may be unfamiliar with the geography of central California).

We undertook this investigation to summarize and clarify the distribution, variation, and taxonomic status of populations of *D. nitratoides.* Herein, we review patterns of morphological differentiation as we examine the adequacy of current subspecies taxonomy. We further add a population genetic perspective derived from mtDNA haplotypes to provide perspective on regional differentiation, population history, and historical patterns of gene flow.

Taxonomic review.—Merriam (1894) described both *nitratoides* and *exilis* as subspecies of the wide-ranging *D. merriami*. Grinnell (1920) described *brevinasus*, noting that the populations of *D. merriami* from the San Joaquin Valley were distinct from other members of that species. In his later review of California kangaroo rats, Grinnell (1922) elevated

nitratoides Merriam to a full species, including within it both *exilis* Merriam and *brevinasus* Grinnell as valid subspecies. Bacular (Best and Schnell 1974), karyotypic (Stock 1971), and allozyme characters (Johnson and Selander 1971; Patton et al. 1976; Best and Janecek 1992) all support the distinctness of *D. nitratoides* relative to *D. merriami*. Alexander and Riddle (2005) confirmed the sister relationship of these two species using mtDNA sequences.

Grinnell (1922) noted that both the Tipton and Fresno kangaroo rats, from the eastern side of the San Joaquin Valley, were dark in overall color tones of the head and dorsum and had dark facial markings. He contrasted both of these races with the paler dorsal tones and facial markings of the short-nosed kangaroo rat and concluded that color differentiation in western populations resulted from adaptation to increasing aridity from east to west due to the rain shadow of the Central Coast Ranges.

Boolootian (1954) studied structural variation in *D. nitratoides*, concluded that *exilis* Merriami did not warrant recognition, and placed it in synonymy of *D. n. nitratoides*. Hall and Kelson (1959) declined to follow Boolootian's (1954) recommendation on the advice of Seth Benson (former Curator of Mammals at the Museum of Vertebrate Zoology, University of California-Berkeley). In a master's thesis on the Fresno kangaroo rat, Hoffmann (1975) concluded that, while Benson erred in his determination of the subspecific allocation of specimens from some localities, *exilis* Merriam was distinct from both *D. n. nitratoides* and *D. n. brevinasus* and concluded that it was, therefore, a valid subspecies. D. F. Williams (in U.S. Fish and Wildlife Service 1988) agreed with Hoffmann's conclusions that the samples Hoffmann regarded as *D. n. exilis* were distinguishable from those he examined of *D. n. nitratoides* and *D. n. brevinasus*. Williams, however, noted that the three subspecies seemed practically indistinguishable when samples of populations from localities intermediate to the geographic locations of Hoffmann's samples were analyzed. Williams et al. (1993) and Patton (2005) provided the most recent reviews of the taxonomy of *D. nitratoides*, both retaining all three subspecies as valid taxa.

Geographic review.—Fresno and Tipton kangaroo rats historically occupied contiguous geographic ranges on the floor of the eastern half of the San Joaquin and Tulare basins in the San Joaquin Valley, respectively (Fig. 1). The short-nosed kangaroo rat occurred in the foothills and basins along the western side of the San Joaquin Valley from about Los Banos, Merced County, southward to the southern and western margins of the Tulare Basin, and in the upper Cuyama Valley and Carrizo Plain (Fig. 1; Grinnell 1933; Williams et al. 1993).

The known historical range of the Fresno kangaroo rat encompassed an area of grassland and chenopod scrub communities on the San Joaquin Valley floor east of the wetlands of the San Joaquin River and Fresno Slough, from the Merced River near Livingston, Merced County, southward to the northern edge of the marshes surrounding Tulare Lake, Kings County, and eastward on the alluvial fans of Sierra Nevada streams (Fig. 1; see also Supplementary Data SD1). The entire historical range was approximately 359,700 ha, although not all of the area would have contained suitable habitat (surveys

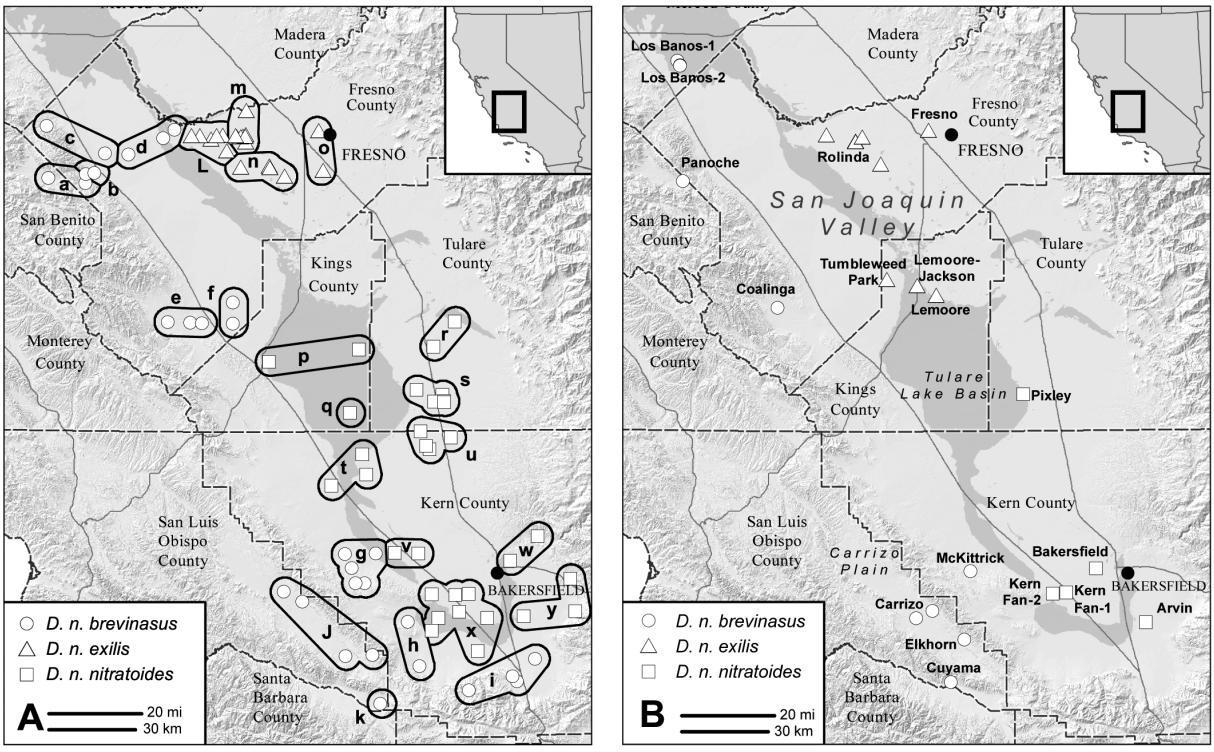

Fig. 1.—(A) Sample localities for the craniodental analyses mapped on the historical (pre-European) land cover of the San Joaquin Valley (dark gray are the historical wetlands, light gray historical shrub and grassland communities; see maps in Supplementary Data SD1 for greater habitat detail); (B) sample localities for the molecular analysis similarly placed on the historical valley land cover.

summarized in Chesemore and Rhodehamel 1992; U.S. Fish and Wildlife Service 1988, 1998). Actual documentation by specimen localities of the historical distribution is, however, scanty. Grinnell (1922:85) simply wrote "so far as known, only a small portion of the east side of the San Joaquin Valley north of Tulare Lake, in the immediate vicinity of Fresno"; he recorded specimens only from Fresno. Culbertson (1934, 1946) and Boolootian (1954) outlined the geographic range as they understood it, and Hoffmann (1975) included specimens only from the vicinities of Fresno, Kerman, and Raisin City, all in Fresno County.

Currently, there are no known populations of the Fresno kangaroo rat within its circumscribed historic range in Merced, Madera, and Fresno counties, though some private properties have never been surveyed. These rats were found on the Alkali Sink Ecological Reserve, west of Kerman, Fresno County in 1981 and 1985, and on adjacent privately owned land in 1981 (Chesemore and Rhodehamel 1992). A single male was trapped on the Reserve in 1992, but attempts in subsequent years failed to find additional animals (U.S. Fish and Wildlife Service 1998). Trapping at other sites in Merced, Madera, Fresno, and Kings counties between 1988 and 2012 also failed to locate other, extant populations within the area regarded as the historical range of the Fresno kangaroo rat (P. A. Kelly, pers. obs.; U.S. Fish and Wildlife Service 1998). The putative Fresno kangaroo rat populations discovered in 1985 on a few undeveloped parcels just south of the historical Kings River course and north of the Tulare Lake bed (Endangered Species Recovery Program [ESRP]—U.S. Fish and Wildlife Service 1998) may now be extirpated. Surveys in 2016 at Tumbleweed Park on Naval Air Station Lemoore, described by Morrison et al. (1996), failed to find evidence of an extant population (B. L. Cypher, pers. obs.).

The historical range of the Tipton kangaroo rat (Fig. 1) was the floor of the Tulare Basin, from approximately the southern margins of Tulare Lake on the north, eastward and southward along the eastern edge of the Valley floor in Tulare and Kern counties, to the foothills of the Tehachapi Mountains, and around the marshes and open water of Kern and Buena Vista lakes and the sloughs and channels of the Kern River alluvial fan. The western margin was approximately along Buena Vista Lake and Buena Vista slough of the Kern River channel into Goose Lake, which periodically emptied into Tulare Lake. D. F. Williams (U.S. Fish and Wildlife Service 1988) estimated this area covered approximately 695,174 ha. Prior to development of water-diversion and irrigation systems subsequent to World War II, the large lake margins and adjoining marshes that predominated in this area were unsuitable as habitat for kangaroo rats (Boolootian 1954), but with diversion of rivers and drainage of these wetlands some areas were subsequently colonized (U.S. Fish and Wildlife Service 1998; U.S. Department of the Interior Interagency Land Retirement Team 2005).

By 1985, the area inhabited by Tipton kangaroo rats had been reduced, primarily by cultivation and urbanization, to about 25,000 ha, approximately 3.6% of the historical range. Additional small, inhabited parcels not surveyed by D. F. Williams (U.S. Fish and Wildlife Service 1988) have since been found, but several of those have been extirpated by development since their discovery. Tipton kangaroo rats have also become reestablished across several hundred to a few thousand hectares of cropland retired since 1985 due to drainage problems or lack of water, or acquired by State and Federal agencies for threatened and endangered species conservation (U.S. Fish and Wildlife Service 1998).

The historical geographic range of short-nosed kangaroo rats is incompletely known from museum and literature records, but the inhabited area was likely greater than 1,000,000 ha. These kangaroo rats occupied the arid grassland and shrubland associations along the western half of the Valley floor and hills on the western edge of the Valley from about Los Banos, Merced County, south to the foothills of the Transverse Ranges on the southern margin of the Valley (Fig. 1). They also occurred on the Carrizo Plain and the upper Cuyama Valley (Grinnell 1922; Boolootian 1954; Williams and Kilburn 1992).

Present occurrences of the short-nosed kangaroo rat are incompletely known because of the lack of comprehensive surveys. Yet relatively intensive livetrapping at several historically occupied sites with extant natural communities indicated that populations were mostly small, fragmented, and widely scattered. These recent efforts found isolated populations in the South Grasslands Waterfowl Unit (Johnson and Clifton 1992; ESRP), Panoche Valley of Fresno and San Benito counties; Cantua Creek, Fresno County; Kettleman Hills, Kings County; Lokern, Elk Hills, San Emigdio, and Wheeler Ridge regions of western and southern Kern County (all summarized in U.S. Fish and Wildlife Service 1998); Carrizo Plain Natural Area, San Luis Obispo County (Vanderbilt-White and White 1992; U.S. Fish and Wildlife Service 1998); and Cuyama Valley, San Luis Obispo County (U.S. Fish and Wildlife Service 1998). Only a few thousand hectares of historical habitat on the San Joaquin Valley floor remain undeveloped. This race occurred on many of the same general areas occupied by the endangered giant kangaroo rat (*Dipodomys ingens*), but with a different pattern of habitat use (Williams 1992; Williams and Kilburn 1992). The extant occupied area is unlikely to be more than about 12,000–15,000 ha, and likely considerably less. The larger estimate represents about 1.5% of the estimated historical habitat (U.S. Fish and Wildlife Service 1998).

Materials and Methods

Craniodental morphometrics.—We examined specimens of *D. nitratoides* in the collections of the Museum of Vertebrate Zoology (MVZ), United States National Museum (USNM), and California State University-Fresno (CSUF), including the holotypes and type series of each subspecies. These collections possess all specimens collected in the late 1800s and early 1900s before the San Joaquin Valley was severely altered by agricultural development. Data are available for a total of 629 specimens, of which 488 were considered adults (defined as specimens in adult pelage and with fully erupted PM4 with some wear) from 79 separate localities (32 short-nosed local-

ities, 19 of Fresno, and 28 of Tipton kangaroo rats; specimens and localities listed in Supplementary Data SD2). All analyses were limited to the adult data set.

We measured 19 craniodental variables using digital calipers at a precision of 0.01 mm. Measurements marked with an asterisk were not ones historically taken on kangaroo rats, but were included because they are dimensions usually accessible on skull fragments typically found in owl pellets, for which no data sets were available. The 19 variables were as follows: greatest skull length (GSL—anterior tip of nasals to posterior expansion of auditory bullae); occipito-nasal length (ONL—anterior tip of nasals to posterior margin of occipital condyles); basilar length (BAL—anterior margin of upper incisors to anterior margin of foramen magnum); nasal length (NL—midline length of nasal bones); nasal width (NW—width of rostrum at suture between premaxilla and maxilla); rostral depth (RD*—depth immediately posterior to upper incisors); length of lacrimal bone (LacL*—lateral distance across lacrimal bone); maxillary breadth (MaxB—greatest distance across maxillary bones); least interorbital distance (IOC—minimum distance across frontal bones between orbits); mastoid breadth (MB—greatest distance across bullae); bullar length (BulL—distance from anterior to posterior margin of auditory bulla); interparietal width (IPW—greatest width of interparietal bone); interbullar width (IBW—greatest width between bullae taken across interparietal bone); diastema length (DL*—posterior margin of upper incisors to anterior margin of PM4); maxillary toothrow length (MTRL—distance from anterior face of PM4 to posterior face of M3 taken at alveolus); alveolar width (AW*—greatest width across the alveolae taken on the outside of the maxillary tooth rows); post-maxillary length (PML*—distance from posterior edge of palate to anterior edge of foramen magnum); basioccipital width (BOW*—distance across distal wings of basioccipital); and cranial depth (CD*—vertical distance from the top of the cranium to the bottom of the tympanic bullae). We obtained the four standard external measurements from specimen labels, including (when available) total length (TOL), tail length (TAL), hind foot length (HFL), and ear length (EL).

We employed univariate and multivariate routines in JMP-Pro (version 14; SAS Institute Inc., Cary, North Carolina) for all morphological analyses, both craniodental and colorimetric. We examined sexual dimorphism in the context of geographic variation by determining the influence of sex on differentiation among the topotypic series of each subspecies for each craniodental variable by two-way analysis of variance (ANOVA, random effects model to accommodate unequal sample sizes). While males were slightly larger in most measurements (1.5% on average), no single, sexually dimorphic univariate variable influenced the degree of differentiation among the subspecies. Consequently, sexes were combined in all craniodental analyses.

We first examined the degree of differentiation among topotypic series of the three subspecies: *D. n. brevinasus* (type locality: Hayes Station, 19 mi SW Mendota, Fresno County; $n = 37$); *D. n. exilis* (type locality: Fresno, Fresno County; $n = 21$); and *D. n. nitratoides* (type locality: Tipton, Tulare County; $n = 51$). We then pooled geographically adjacent localities assigned to the same subspecies into 25 groups (listed in Supplementary Data SD2) to assess geographic trends across five separate transects: 1) Northern transect—short-nosed and Fresno kangaroo rats, including their respective topotypic series, from San Benito and Fresno counties; 2) Central transect—short-nosed and Tipton kangaroo rats, including the topotypic series of the latter, from southern Fresno, Kings, and western Tulare counties; 3) Southern transect—short-nosed and Tipton kangaroo rat samples from the Cuyama and Carrizo basins east to the foothills of the Tehachapi Mountains, including topotypic series of the former; 4) Eastern transect—Fresno and Tipton kangaroo rats along the east side of the San Joaquin Valley from Fresno to Kern County, including topotypic series of both; and 5) Western transect—short-nosed kangaroo rat samples along the western margins of the San Joaquin Valley from San Benito-Fresno counties in the north to southern Kern County in the south. Samples with fewer than five specimens were not included in the transect analyses. In each analysis, we tested for size and combined craniodental variable differences among the suite of samples compared. We estimated general size from scores on the first principal components (PC) axis, as these values correlated strongly with greatest skull length (R^2 ranged from 0.796 [Western transect] to 0.859 [Northern transect], $P < 0.001$ in each case). We used standardized scores on the first canonical variates (CV) axis to summarize combined variable differences among the samples being compared; other CV axes provided significant differences among samples, but these did not appreciably change the patterns of among-sample differentiation obtained from CV-1 alone. Both subspecies topotypic series and grouped locality comparisons used the Tukey–Kramer post hoc test derived from one-way ANOVAs, with a Bonferroni correction applied for multiple tests, to test the null hypothesis of statistical homogeneity among samples. All multivariate analyses used $\log_{10}$ transformations of the original cranial variables. In each analysis, we assigned subspecies to these pooled samples based on the historically defined geographic boundaries described above and mapped in Fig. 1.

Colorimetric analyses.—We measured color reflectance with an X-Rite Digital Swatchbook spectrophotometer (X-Rite, Inc., Grandville, Michigan) on the mid-dorsum of 322 kangaroo rat specimens (specimens and localities listed in Supplementary Data SD3). Data were limited only to specimens in the MVZ collections. The instrument was set to the CIE (Commission Internationale de l'Eclairage) Standard Illuminant F7 for fluorescent illumination, which represents a broadband daylight fluorescent lamp (6,500 K). We chose this standard because all color reflectance was measured indoors under fluorescent ambient lighting. The instrument provides a reflectance spectrum (390–700 nm) of the object being measured as well as tristimulus color scores (CIE X, Y, and Z) that can be directly compared to scores from the Munsell, or other color references (e.g., Hill 1998). We included only adult specimens with nonoily fur. The trichromatic X, Y, and Z values were highly correlated with Pearson product-moment correlation coeffi-

cients > 0.874 and *P*-values < 0.001 (Fisher's *Z*-test) in each comparison. Consequently, we employed a principal components analysis (PCA) on the original variables and used the resulting PC-1 scores in all colorimetric comparisons among subspecies and population samples. Tukey–Kramer post hoc tests, with a Bonferroni correction, were used to test the null hypothesis of the lack of differentiation among samples.

Molecular analyses.—We generated sequence data from a 405-bp fragment of the mitochondrial cytochrome *b* (*Cytb*) gene, beginning with the start codon, from 218 specimens. Locality data were pooled into 18 population samples (see Supplementary Data SD4 for sample localities and specimens) that included five Tipton (n = 60), two Fresno (n = 14), and eight short-nosed (n = 110) kangaroo rat groupings. We also have samples from three populations from northern Kings County (n = 34) within the historic range of the Fresno kangaroo rat but for which subspecies identity could not be validated as no vouchers were preserved. These are referred to as putative Fresno kangaroo rats. We obtained sequences from both samples of the Fresno (12 individuals from the vicinity of Rolinda and Kerman from the collection at CSU-Fresno and two individuals of the original type series from Fresno now in the MVZ) and one sample of short-nosed kangaroo rats (six specimens from Panoche, also in the CSU-Fresno collection) from skin snips of museum specimens. All other samples were either ear biopsies taken from marked and released kangaroo rats as part of longitudinal population studies or tissues preserved at the time specimens were collected and subsequently archived in the MVZ. Sample sizes range from 2 (Fresno) to 26 (Los Banos), for an average of 12 specimens per population; 12 of the 18 pooled samples comprised 10 or more individuals.

We gathered data over a 17-year period. As a result, methods of DNA extraction, PCR amplification, and sequencing varied substantially as technology changed during this time frame. Our initial methods were those outlined in Smith and Patton (1991, 1993), which were based on a sodium dodecyl sulfate-proteinase K–phenol–RNase extraction method (Maniatis et al. 1982) with double-stranded PCR using primer pairs MVZ05–MVZ04 followed by asymmetric single-strand amplification, with sequences obtained using manual sequencing methods on acrylamide slab gels and S^{35} visualization. For more recent data, we used the methods outlined in Patton et al. (2008) with an ABI3730 capillary automated sequencer following manufacturer's protocols after purification of the double-stranded DNA using the QIAquick PCR kit (Qiagen, Valencia, California), and then cycle-sequenced this template with primer MVZ05. Sequences were either read by hand or aligned using the Sequence Navigator software (Applied Biosystems, Inc., Foster City, CA). For sequences obtained from museum specimens, we followed established guidelines for "ancient" DNA (e.g., Gilbert et al. 2005). We removed a small piece of skin from the edge of the ventral incision with sterilized instruments, carefully removed hair with a sterile scalpel blade, and then soaked the skin fragment in sterile ddH_2O overnight followed by extraction using DNAeasy kits (Qiagen). All procedures took place in a DNA clean room under a positive pressure hood to minimize opportunities for contamination from external airflow.

We identified redundant sequences using Collapse, version 1.2, and constructed a parsimony network depicting genealogical relationships among the unique haplotypes using TCS, version 1.21 (both software packages of David Posada and obtainable at http://darwin.uvigo.es/). We tested the patterns of haplotype distribution and differentiation to determine if they were reflective of an historical signature either of long-term stability, population expansion, or population decline by calculating Tajima's *D* and Fu's *Fs* values. We also calculated mismatch distributions, and performed a nested analysis of molecular variance (AMOVA) in the Arlequin 3.1 package (Excoffier et al. 2005). We calculated the population genetic measure Θ, an estimate of the effective population size, from the relationship $\Theta = 2N_f\mu$ (where N_f = the number of females, for mtDNA genes, and μ is the mutation rate—Tajima 1989). The estimate Θ_k, based on the average number of pairwise differences (Tajima 1989), reflects current population sizes versus the effective population size over historic time (Good et al. 1997).

We employed the Bayesian approach implemented in BEAST (Drummond et al. 2006; Drummond and Rambaut 2007) to examine the demographic history of *D. nitrotoides*. For this analysis, we included *Cytb* sequences from GenBank for *D. deserti* (as outgroup; AY926381), *D. phillipsi* (AY926378), *D. merriami* (AF173502, AF172837, EU661056, EU661020, EU661021, and EU661026), and the 43 unique haplotypes of *D. nitratoides* to establish the base of the *D. merriami–D. nitratoides* divergence. We estimated the time for each node using the 15.4 Ma mean date for the base of *Dipodomys* from Hafner et al. (2007), with an estimated standard deviation of 1.0 Ma, an HKY substitution model with empirical base frequencies and gamma + invariate sites heterogeneity, and under an uncorrelated lognormal relaxed clock (which assumes independent rates on different branches and no a priori correlation between a given lineage's age and that of its ancestor) and the Yule model of speciation (which assumes a constant speciation rate per lineage). We ran the analysis with Markov chain Monte Carlo (MCMC) options set to 3,000,000 chains sampled at intervals of 200, building the final tree after a burn-in of 1,000, and under both constant and linear growth models.

Research on live animals followed ASM guidelines (Sikes et al. 2016) and was approved by the Animal Welfare Committee of California State University, Stanislaus. We submitted all sequences to GenBank (accession numbers MN087818–MN088034).

Results

Morphological differentiation.—Statistical comparisons of topotypes of each subspecies for three external, 19 craniodental, and three color variables support Grinnell's (1920, 1933) conclusions that the Fresno kangaroo rat was remarkable for its overall small size and dark color tones, the Tipton kangaroo rat was somewhat larger with a similar darkened color, and the

short-nosed kangaroo rat was the largest in nearly all dimensions and with a paler color. The Fresno kangaroo rat was significantly smaller than both other subspecies in 16–19 of 22 mensural variables (TOL, TAL, GSL, BAL, NL, NW, LacL, MaxB, IOC, MB, BulL, DL, AW, PML, BOW, and CD between Fresno and Tipton; TOL, TAL, HF, GSL, ONL, BAL, NL, NW, RD, LacL, MaxB, IOC, MB, BulL, DL, AW, PML, BOW, and CD between Fresno and short-nosed; see Table 1) and the Tipton kangaroo rat was smaller than the short-nosed in 12 of the 22 variables (TOL, TAL, HF, GSL, NL, RD, MaxB, IOC, MB, BulL, DL, and PML). The three topotypic series, unsurprisingly, differ in general size (PC-1 scores) and canonical combinations of craniodental variables (CV-1 scores; Tukey–Kramer pairwise $P < 0.001$ in each comparison for both data sets; PC-1 and CV-1 eigenvalues provided in Table 2). The Fresno kangaroo rat is also the darkest of the three subspecies, Tipton is intermediate in color tones, and short-nosed are palest (pairwise comparisons are significant, with $P < 0.001$ in each case).

These results are consistent with descriptions provided by Merriam (1894) and Grinnell (1920) in their respective diagnoses of the three taxa, and also confirm Grinnell's (1933) assertion that the bullae of short-nosed kangaroo rats are more inflated compared with either Fresno or Tipton kangaroo rats (pairwise Tukey–Kramer post hoc tests, $P \leq 0.001$). However, differences between the type series address neither the extent to which there is geographic variation within each taxon nor the geographic pattern across the entire distributional range should such variation exist. Nor can topotypic comparisons alone confirm the historic subspecies boundaries as delimited by Grinnell (1922, 1933), Hoffmann (1975), and Williams et al. (1993). To address these issues, we examined patterns of differentiation for each data set (craniodental PC-1 scores for general size, craniodental CV-1 scores, and color PC-1 scores; eigenvalues provided in Tables 2 and 3) across each of five transects (Northern transect, which connects samples of short-nosed and Fresno kangaroo rats; Central transect, which connects short-nosed and Tipton kangaroo rats; Southern transect, which also compares short-nosed and Tipton samples; Eastern transect, along the eastern side of the San Joaquin Valley that includes Fresno and Tipton samples; and Western transect, along the western side of the Valley of short-nosed samples alone).

General size trends along three transects (Northern, Central, and Eastern) are consistent with both the original subspecies diagnoses and the historically placed boundaries between them (Fig. 2, upper left, upper central, and lower right panels). For both the Northern transect and Central transects, the individual samples of short-nosed kangaroo rats and those of either Fresno and Tipton kangaroo rats each form minimally nonsignificant subsets that differ substantially (Tukey–Kramer post hoc $P < 0.001$) between the subspecies pairs, with short-nosed samples uniformly larger in both sets of comparisons. A similar pattern of distinction (again where Tukey–Kramer post hoc $P < 0.001$) held for the Eastern transect, where Fresno kangaroo rats exhibited uniform, but smaller sizes, across their sampled populations than the uniformly larger Tipton kangaroo rat samples. In contrast, the Western transect of short-nosed samples (Fig. 2, lower left panel) exhibited uniform size from north to south, and the Southern transect (Fig. 2, upper right panel), which connected short-nosed and Tipton kangaroo rats at their southernmost distribution, also failed to differentiate between the two subspecies.

Although the Tipton kangaroo rat samples form a single, nonsignificant subset (see Eastern transect, Fig. 2), it is noteworthy that northern Tipton samples (p-Tulare Lake, q-Corcoran, r-Tipton, and s-Earlimart) average slightly smaller than southern ones (w-Bakersfield, x-Buena Vista Lake, and y-Arvin). When the four southernmost short-nosed samples (j-Carrizo, g-McKittrick, k-Cuyama, and i-San Emigdio) are compared to the four northernmost Tipton samples, their PC-1 scores differ significantly ($P < 0.001$), with Tipton kangaroo rats smaller in general size.

In dorsal color tones, all short-nosed samples were significantly paler in comparison to either Fresno or Tipton samples in the Northern and Central transects, respectively (Fig. 3, upper left and upper central panels) but uniformly pale across their range (Western transect, Fig. 3, lower left panel). In the Northern transect, however, the statistical break between adjacent samples was to the east of where it was geographically placed in general size (Fig. 2), as the westernmost Kerman sample (l-Kerman) of the Fresno kangaroo rat was indistinguishable from short-nosed samples in paleness. As a result, Fresno kangaroo rats, but only the topotypic series from Fresno (sample o), are significantly darker than both short-nosed kangaroo rats and all Tipton kangaroo rat samples further to the south (Eastern transect, lower right panel, Fig. 2; Tukey–Kramer post hoc $P < 0.001$). In the Southern transect, exclusive of sample i-San Emigdio, which forms a statistical bridge between short-nosed and Tipton samples (Fig. 3, upper right panel), samples g-McKittrick and j-Carrizo are sharply differentiated from Tipton samples w-Bakersfield, y-Arvin, and x-Buena Vista Lake (Tukey–Kramer post hoc $P < 0.001$).

Geographic trends also occur with respect to the multivariate synthesis of craniodental traits, as depicted by CV-1 scores, but in somewhat more complex patterns than either general size or color (Fig. 4). Samples across the range of the short-nosed kangaroo rat are, however, homogeneous, even if comprised of two broadly overlapping but statistically different minimally cohesive sets of samples (Fig. 4, lower left panel; Tukey–Kramer post hoc $P = 0.01$ between samples b-Panoche and k-Cuyama). With the exception of the Eastern transect, where samples of both the Fresno and Tipton kangaroo rats are statistically homogeneous within but strongly differentiated between their respective samples (Fig. 4, lower right panel; Tukey–Kramer host-hoc $P < 0.001$), the other transects comparing samples belonging to different subspecies exhibit step clines but with breaks discordantly positioned with respect to subspecies boundaries. For example, in the Northern transect (Fig. 4, upper left panel), the post hoc break ($P < 0.001$) occurs between the o-Fresno sample and all others, with the more northern and western samples of the Fresno kangaroo rat (l-Kerman-W, m-Kerman, and n-Rolinda) indistinguishable from short-nosed samples. In the Central transect, the short-nosed sample f-Huron bridges the statistically

Table 1.—Descriptive statistics (mean, standard error, range, and sample size) of three external, 19 craniodental variables, and three color variables for topotypic series of each subspecies of the San Joaquin kangaroo rat, *Dipodomys nitratoides*. Pairwise *P*-values derived from Tukey–Kramer post hoc tests following one-way analyses of variance (ANOVAs) are given in the columns between subspecies character values: * comparison between Tipton and Fresno samples; ** above, comparison between Fresno and short-nosed; below, comparison between Tipton and short-nosed.

Variable	Tipton	*P*-value*	Fresno	*P*-value**	Short-nosed
TOL	231.3 ± 1.29 212–253 *n* = 49	*P* = 0.02	224.5 ± 2.04 211–245 *n* = 20	*P* = 0.001/*P* = 0.001	243.3 ± 1.39 221–251 *n* = 36
TAL	137.4 ± 1.21 114–152 *n* = 49	*P* = 0.09	132.5 ± 1.55 122–147 *n* = 20	*P* = 0.001/*P* = 0.03	141.5 ± 1.13 125–155 *n* = 36
HF	34.4 ± 0.15 31–37 *n* = 52	*P* = 0.05	33.8 ± 0.17 33–35 *n* = 20	*P* = 0.001/*P* < 0.001	35.7 ± 0.13 34–38 *n* = 37
GSL	33.55±0.13 31.0–35.2 *n* = 51	*P* < 0.001	32.01 ± 0.25 30.5–33.6 *n* = 21	*P* < 0.001/*P* < 0.001	34.49 ± 0.15 30.82–39.98 *n* = 37
ONL	30.30 ± 0.15 27.9–33.0 *n* = 51	*P* = 0.17	29.86 ± 0.28 27.5–31.5 *n* = 21	*P* = 0.02/*P* = 0.23	30.64 ± 0.10 29.3–31.8 *n* = 37
BAL	23.43 ± 0.17 21.4–27.1 *n* = 51	*P* = 0.001	22.26 ± 0.205 21.1–23.7 *n* = 21	*P* < 0.001/*P* = 0.07	26.9 ± 0.13 22.9–26.9 *n* = 37
NL	11.92 ± 0.07 10.4–12.8 *n* = 51	*P* < 0.001	11.20 ± 0.10 10.6–12.1 *n* = 21	*P* < 0.001/*P* = 0.004	12.28 ± 0.51 11.4–13.9 *n* = 37
NW	2.99 ± 0.03 2.5–3.4 *n* = 51	*P* = 0.02	2.84 ± 0.05 2.2–3.4 *n* = 21	*P* = 0.001/*P* = 0.08	3.08 ± 0.03 2.8–3.4 *n* = 37
RD	5.79 ± 0.03 5.3–6.2 *n* = 51	*P* = 0.06	5.66 ± 0.05 5.4–6.1 *n* = 21	*P* < 0.001/*P* = 0.001	5.96 ± 0.04 5.5–6.4 *n* = 37
LacL	3.02± 0.03 2.5–3.4 *n* = 51		2.76 ± 0.06 2.3–3.4 *n* = 21	*P* = 0.001/*P* = 0.84	3.05 ± 0.04 2.27–3.84 *n* = 37
MaxB	18.42 ± 0.07 16.9–19.3 *n* = 51	*P* < 0.001	17.33 ± 0.156 16.2–18.5 *n* = 21	*P* < 0.001/*P* < 0.001	19.04±0.09 17.8–19.5 *n* = 37
IOC	11.84 ± 0.07 10.6–13.3 *n* = 51	*P* = 0.003	11.38 ± 0.08 10.8–11.9 *n* = 21	*P* < 0.001/*P* = 0.02	12.13 ± 0.08 11.1–13.5 *n* = 37
MB	21.88 ± 0.08 20.9–23.3 *n* = 51	*P* < 0.001	20.54 ± 0.17 19.5–21.7 *n* = 21	*P* < 0.001/*P* = 0.001	22.42 ± 0.08 21.3–23.9 *n* = 37
BulL	14.45 ± 0.08 12.9–15.3 *n* = 51	*P* < 0.001	13.67 ± 0.12 12.4–15.5 *n* = 21	*P* < 0.001/*P* = 0.001	14.86 ± 0.08 13.8–15.9 *n* = 37
IPW	1.67 ± 0.05 1.0–2.4 *n* = 51	*P* = 0.55	1.76 ± 0.08 1.0–2.3 *n* = 21	*P* = 0.42/*P* = 0.94	1.65 ± 0.06 0.9–2.5 *n* = 37
IBW	2.29 ± 0.06 1.5–3.3 *n* = 51	*P* = 0.33	2.12 ± 0.08 1.5–3.7 *n* = 21	*P* = 0.13/*P* = 0.71	2.37 ± 0.07 1.4–3.5 *n* = 37
DL	7.47 ± 0.04 6.7–8.0 *n* = 51	*P* = 0.005	7.21 ± 0.071 6.7–7.8 *n* = 21	*P* < 0.001/*P* = 0.001	7.75±0.05 7.2–8.3 *n* = 37
MTRL	4.13 ± 0.04 3.5–5.0 *n* = 51	*P* = 0.73	4.08 ± 0.06 3.7–4.6 *n* = 21	*P* = 0.64/*P* = 0.97	4.14 ± 0.04 3.7–4.7 *n* = 37
AW	6.59 ± 0.03 6.1–7.2 *n* = 51	*P* < 0.001	6.13 ± 0.05 5.8–6.4 *n* = 21	*P* < 0.001/*P* = 0.57	6.63 ± 0.03 6.2–7.1 *n* = 37
PML	12.64 ± 0.07 10.4–13.7 *n* = 51	*P* < 0.001	11.94 ± 0.12 10.9–13.0 *n* = 21	*P* < 0.001/*P* = 0.001	13.07 ± 0.08 12.1–14.1 *n* = 37

Table 1.—Continued

Variable	Tipton	*P*-value*	Fresno	*P*-value**	Short-nosed
BOW	5.07 ± 0.04 4.6–5.7 *n* = 51	*P* = 0.001	4.75 ± 0.05 4.4–5.1 *n* = 21	*P* < 0.001/*P* = 0.52	5.14 ± 0.06 4.0–5.7 *n* = 37
CD	10.71 ± 0.06 9.7–11.5 *n* = 51	*P* < 0.001	9.9 ± 0.17 9.1–11.0 *n* = 21	*P* < 0.001/*P* = 0.75	10.80 ± 0.05 10.0–11.5 *n* = 37
Color-X	8.7 ± 0.16 7.1–9.4 *n* = 17	*P* = 0.008	7.29 ± 0.71 5.7–8.8 *n* = 4	*P* < 0.001/*P* < 0.001	12.37 ± 0.37 10.2–14.4 *n* = 14
Color-Y	8.7 ± 0.16 7.0–9.5 *n* = 17	*P* = 0.11	7.35 ± 0.75 5.6–9.0 *n* = 4	*P* < 0.001/*P* < 0.001	12.35 ± 0.38 10.3–14.7 *n* = 14
Color-Z	5.47 ± 0.15 4.6–7.2 *n* = 17	*P* = 0.87	5.21 ± 0.59 3.7–6.3 *n* = 4	*P* < 0.001/*P* < 0.001	8.34 ± 0.31 5.7–10.2 *n* = 14

significant gap between short-nosed e-Mendota and the four samples of Tipton kangaroo rats (Fig. 4, upper middle panel; Tukey–Kramer post hoc $P < 0.001$). And, in the Southern transect, the geographically intermediate Tipton (v-Buttonwillow and x-Buena Vista Lake) and short-nosed (i-San Emigdio) samples form a cline connecting otherwise homogenous western short-nosed samples (j-Carrizo, g-McKittrick, and k-Cuyama) and easternmost Tipton samples (w-Bakersfield and y-Arvin), but with the major break between Tipton v-Buttonwillow and w-Bakersfield.

Overall, differences in general size and color are consistent with the original subspecies descriptions and mirror the geographic pattern expected from the historically drawn subspecies boundaries, although with two minor caveats for color trends: similarity between the Kerman sample of the Fresno kangaroo rats with adjacent short-nosed populations, and the intermediate color of the San Emigdio sample with respect to short-nosed and Tipton samples. Geographic trends in multivariate craniodental characters, as mentioned, are both more complex and nuanced but still generally concordant with geographic expectations. This is particularly true for short-nosed–Tipton and Fresno–Tipton comparisons. One potentially substantive caveat with regard to the graphical depiction represented by CV-1 scores alone (Fig. 4) is that the pool of variation explained by this axis accounts for only 40% to 70% of the total in the separate analyses (Fig. 4; Table 2). Accounting for variation on other axes simultaneous with that on CV-1 would be preferable, if possible.

Molecular differentiation.—There are 43 haplotypes among the 218 individuals sampled across 18 populations, the major-

Table 2.—Canonical variate (CV-1) and principal component (PC-1) eigenvectors for 19 $\log_{10}$-transformed craniodental variables for each of the six analyses of the San Joaquin kangaroo rat, *Dipodomys nitratoides*: topotypic series, Northern transect (short-nosed versus Fresno); Central transect (short-nosed versus Tipton); Southern transect (short-nosed versus Tipton); Eastern transect (Fresno versus Tipton); and Western transect (short-nosed only). The eigenvalue and percent contribution for each axis are listed at the bottom.

Variable	Type series		Northern transect		Central transect		Southern transect		Eastern transect		Western transect	
	CV-1	PC-1	CV-1	PC-1	CV-1	PC-1	CV-1	PC-1	CV-1	PC-1	CV-1	PC-1
$\log_{10}$GSL	0.153	0.306	0.580	0.292	–0.039	0.375	–0.001	0.327	–0.001	0.327	–0.561	0.380
$\log_{10}$ONL	–0.080	0.215	–0.362	0.255	–0.970	0.299	–0.091	0.251	–0.091	0.251	0.407	0.266
$\log_{10}$BAL	0.181	0.243	–0.016	0.277	0.153	0.244	0.103	0.259	0.103	0.259	–0.771	0.333
$\log_{10}$NL	0.135	0.276	–0.063	0.280	0.308	0.206	–0.085	0.288	–0.085	0.288	0.622	0.322
$\log_{10}$NW	–0.092	0.195	0.068	0.165	–0.008	0.221	–0.259	0.153	–0.259	0.153	–0.483	0.163
$\log_{10}$RD	–0.326	0.247	–0.068	0.262	–0.153	0.293	0.183	0.272	0.183	0.272	–0.081	0.265
$\log_{10}$LacL	0.036	0.146	0.098	0.188	0.116	0.186	0.020	0.158	0.020	0.158	0.054	0.107
$\log_{10}$MaxB	0.281	0.281	0.104	0.270	0.069	0.264	–0.009	0.267	–0.009	0.267	–0.66	0.256
$\log_{10}$IOC	–0.033	0.208	0.161	0.225	–0.350	0.309	0.006	0.200	0.006	0.200	0.225	0.181
$\log_{10}$MB	0.446	0.298	0.484	0.286	0.126	0.098	0.490	0.306	0.490	0.306	0.133	0.249
$\log_{10}$BuL	–0.052	0.263	–0.247	0.270	0.225	0.279	0.091	0.283	0.091	0.283	0.465	0.269
$\log_{10}$IPW	0.034	–0.081	–0.329	–0.123	–0.382	–0.094	–0.106	–0.117	–0.106	–0.117	–0.047	–0.067
$\log_{10}$IBW	0.168	0.016	0.271	0.061	0.539	–0.092	0.224	0.014	0.224	0.014	–0.385	–0.074
$\log_{10}$DL	0.038	0.261	–0.037	0.246	0.428	0.043	–0.111	0.209	–0.111	0.209	0.327	0.244
$\log_{10}$MTRL	–0.361	0.114	–0.010	0.176	–0.203	0.151	0.237	0.175	0.237	0.175	0.383	0.137
$\log_{10}$AW	0.426	0.255	0.137	0.209	0.155	0.124	–0.027	0.209	–0.027	0.209	0.480	0.123
$\log_{10}$PML	0.145	0.281	0.129	0.284	–0.124	0.144	0.247	0.294	0.247	0.294	–0.217	0.317
$\log_{10}$BOW	0.103	0.206	0.229	0.212	0.321	0.289	0.357	0.227	0.357	0.227	0.063	0.148
$\log_{10}$CD	0.196	0.224	0.181	0.084	0.048	0.291	–0.665	0.050	–0.665	0.050	0.017	0.059
Eigenvalue	3.214	8.754	3.017	10.014	1.192	6.035	1.622	7.768	1.622	7.768	1.893	5.980
%	90.7	46.1	53.7	52.7	39.9	31.8	71.5	40.9	71.5	40.9	39.6	31.5

Table 3.—Principal component (PC-1) scores for three color variables for each of the six analyses of the San Joaquin kangaroo rat, *Dipodomys nitratoides*: topotypic series, Northern transect (short-nosed versus Fresno); Central transect (short-nosed versus Tipton); Southern transect (short-nosed versus Tipton); Eastern transect (Fresno versus Tipton); and Western transect (short-nosed only). The eigenvalue and percent contribution for each axis are listed at the bottom.

Variable	Type series	Northern transect	Central transect	Southern transect	Eastern transect	Western transect
	PC-1	PC-1	PC-1	PC-1	PC-1	PC-1
X	0.583	0.584	0.604	0.583	0.588	0.583
Y	0.587	0.589	0.632	0.587	0.596	0.587
Z	0.561	0.558	0.486	0.562	0.547	0.562
Eigenvalue	2.846	2.826	2.424	2.853	2.742	2.854
%	94.9	94.2	80.8	95.1	91.4	95.1

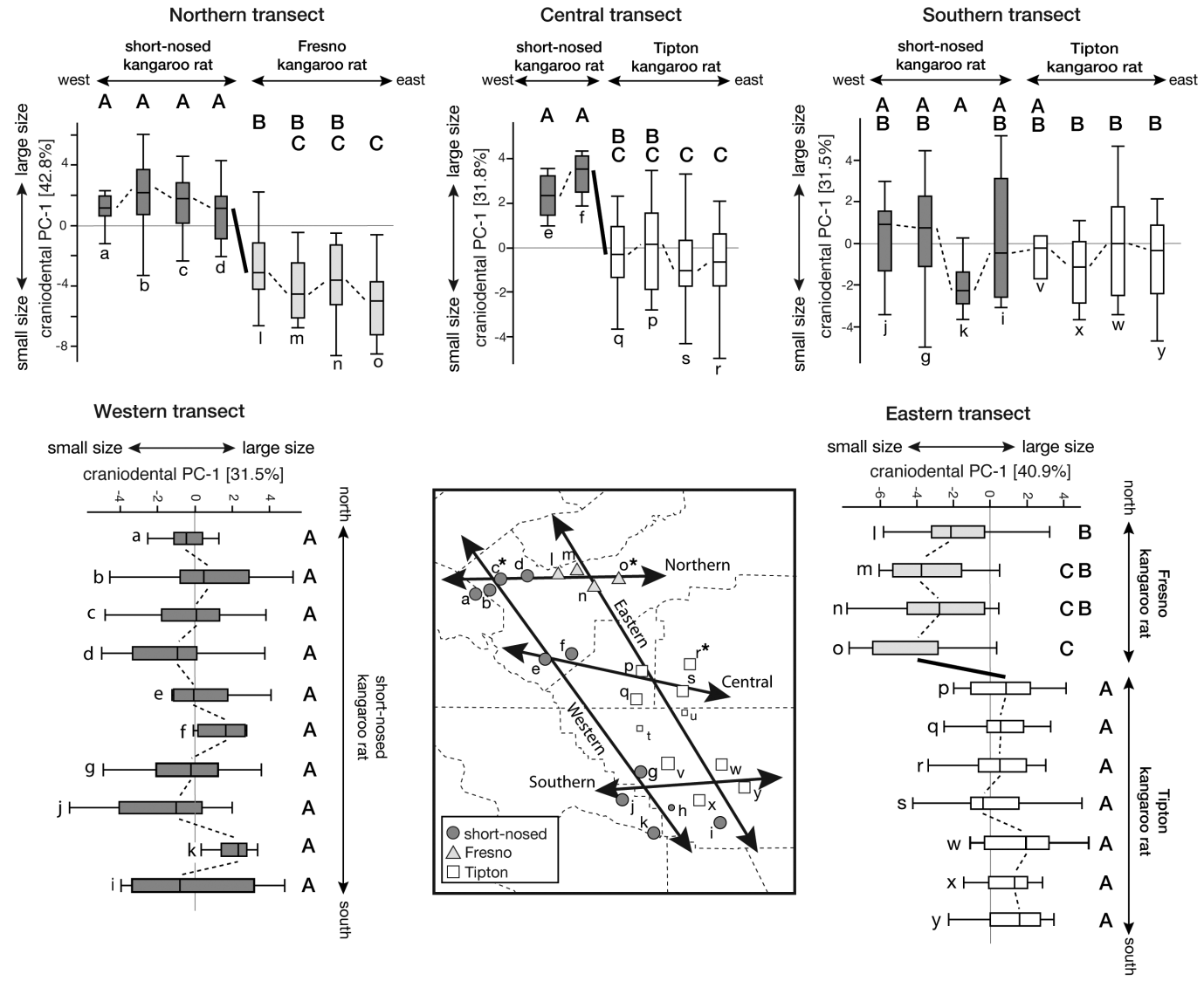

Fig. 2.—Box plots of principal component (PC-1) scores representing general size variation across each of the five transects described in the text and identified in Fig. 1 and the map of included localities, below middle. Size shifts from smaller to larger from negative to positive PC-1 values. Different gray tones identify samples from each of the three subspecies. Tukey–Kramer minimally significant subsets are indicated by the capital letters above each set of box plots; dashed lines indicate nonsignificant differences between adjacent samples (where pairwise $P > 0.05$) and bold black lines identify significant breaks (where pairwise $P < 0.001$) in the set of samples compared along each transect.

ity of which (35) are limited to a single subspecies (18 short-nosed; 11 Tipton; and six Fresno kangaroo rats [including those putatively Fresno]; Table 4). The three subspecies have similar and nonsignificant levels of haplotype and nucleotide diversity; the former measure is lowest in the putative Fresno kangaroo rat samples from Lemoore and Tumbleweed. Not surprisingly,

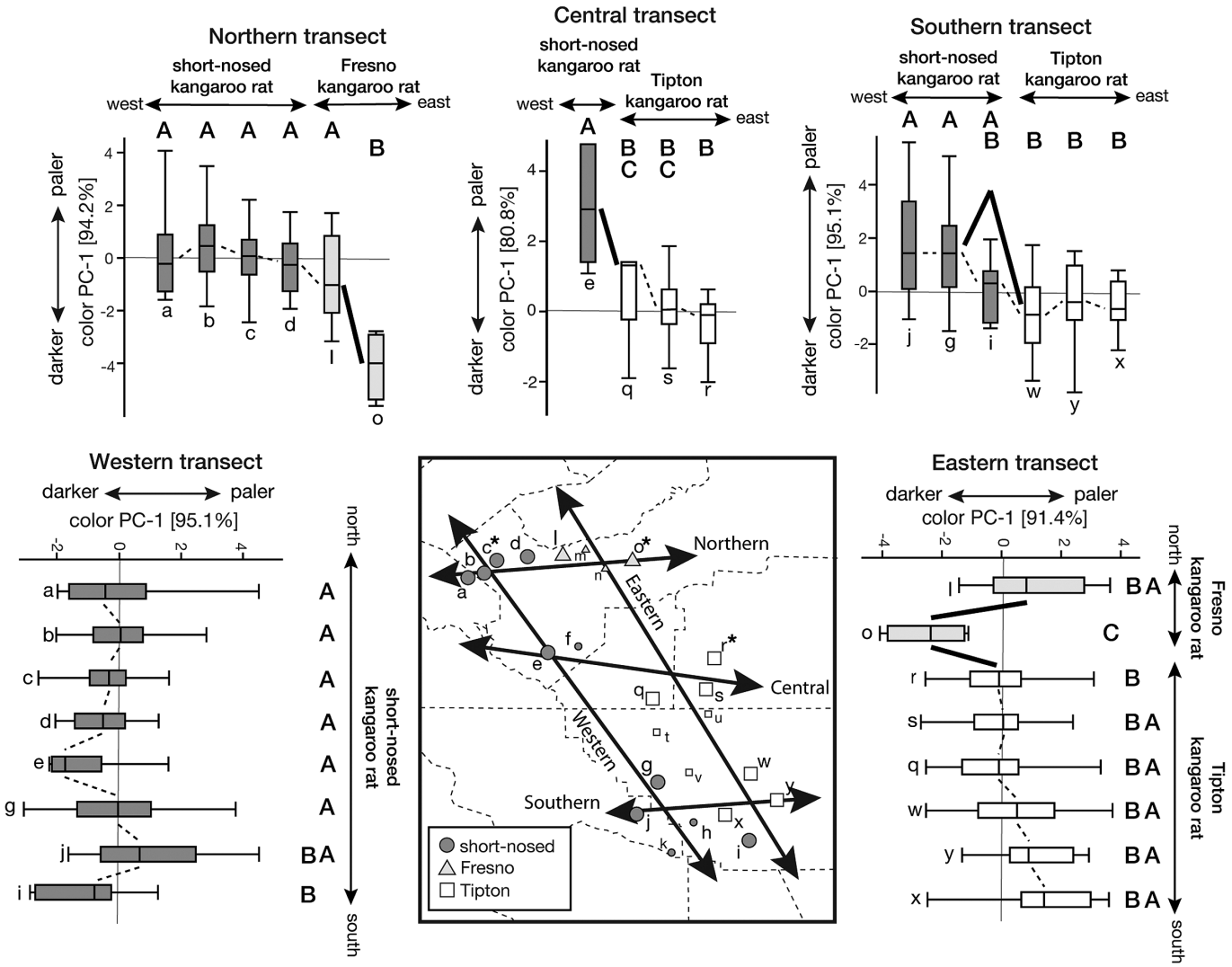

Fig. 3.—Box plots of principal component (PC-1) scores representing dorsal color variation across each of the five transects described in the text and identified in Fig. 1 and the map of included localities, below middle. Negative to positive PC-1 values identify a shift in color tones from darker to paler. Different gray tones identify samples from each of the three subspecies. Kramer minimally significant subsets are indicated by the capital letters above each set of box plots; dashed lines indicate nonsignificant differences between adjacent samples (where pairwise $P > 0.05$) and bold black lines identify significant breaks (where pairwise $P < 0.001$) in the set of samples compared along each transect.

both measures vary across the individual samples, both within and among the subspecies. All 11 individuals examined in the Jackson Avenue sample of what is presumed to be the Fresno kangaroo rat had the same haplotype. This contrasts with the Fresno (n = 2) and Panoche (n = 6) samples for which each sampled individual had a unique haplotype (haplotype diversity = 1.00; nucleotide diversity 0.0074 and 0.0058, respectively). In general, however, there is reasonable haplotype diversity in nearly all populations, perhaps unexpectedly so given the fragmented nature of current populations. The number of haplotypes within populations is not related to the sample size ($R^2 = 0.024$, $F_{1,16} = 0.396$, $P = 0.538$).

All haplotypes are closely related, largely separated by single-mutational steps. The mean p-distance in the comparison of all haplotypes is only 0.0104 (range 0.0025–0.0222). This remarkable uniformity of overall molecular similarity within *D. nitratoides* is mirrored by comparisons of haplotypes found in each subspecies, where mean uncorrected p-distances range from a low of 0.0092 for Fresno samples to 0.0096 for Tipton samples. These slight differences among subspecies are not significant (ANOVA, $P > 0.05$ in each pairwise comparison).

The lack of differentiation among as well as within subspecies of the San Joaquin kangaroo rat is readily apparent in the parsimony network (Fig. 5) of sampled haplotypes. All but five (or six based on alternative pathways in the network) haplotypes are single steps from their nearest neighbor among those recovered, and only five unsampled (either now extinct or simply undetected in the available samples) haplotypes are hypothesized. All but three haplotypes unique to a subspecies are in low frequency (found in fewer than five individuals). Eight haplotypes are shared among subspecies, two between Fresno and short-nosed populations, three between short-nosed and Tipton, and three between all three subspecies. Shared haplotypes, especially those shared among all three subspecies, are

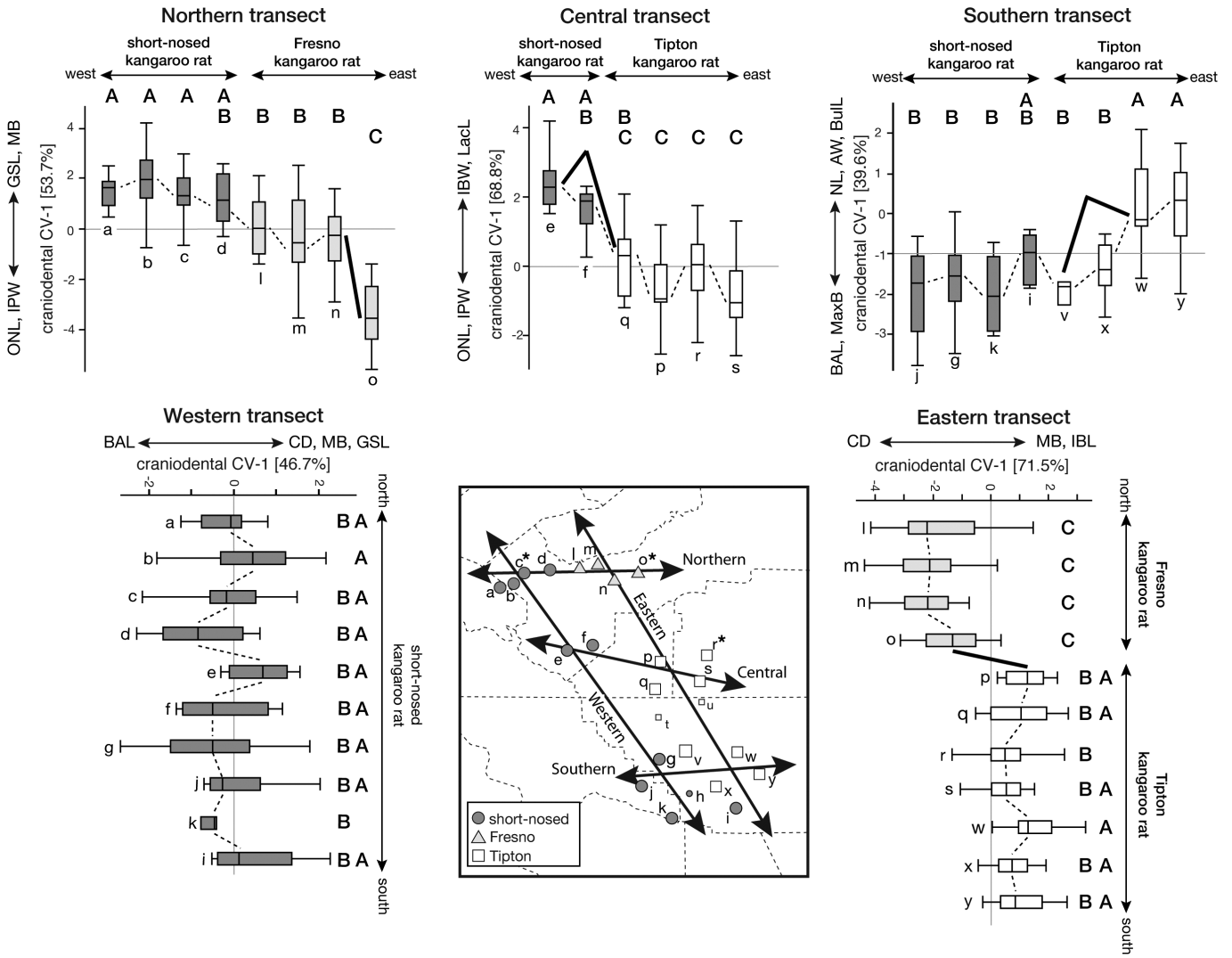

Fig. 4.—Box plots of canonical variate (CV-1) scores representing the major axis of multivariate combinations of craniodental variables across each of the five transects described in the text and identified in Fig. 1 and the map of included localities, below middle. Variables with the highest loadings (see Table 2) are identified. Different gray tones identify samples from each of the three subspecies. Kramer minimally significant subsets are indicated by the capital letters above each set of box plots; dashed lines indicate nonsignificant differences between adjacent samples (where pairwise $P > 0.05$) and bold black lines identify significant breaks (where pairwise $P < 0.001$) in the set of samples compared along each transect.

the most common (five haplotypes [2, 4, 16, 20, and 28] are found in 44% of all individuals examined [96 out of 218]). A nested AMOVA also fails to support a "subspecies effect" in the apportionment of haplotype variation. Only 1.99% of the total pool of variation can be attributed to differences among subspecies and 26.67% to differences among samples within subspecies. The majority of variation, 71.34%, is that within populations. There is also no relationship between haplotype diversity and geography; a Mantel test of the matrix correlation between $\log_{10}(F_{ST}$, a measure of population differentiation) and $\log_{10}$(geographic distance) is nonsignificant ($R^2 = 0.001$, $Z = 0.067$, $P = 0.796$).

Fu's *Fs* and Tajima's *D* statistics (Table 5), which measure deviations from neutral expectations, are not significantly different from zero, with six exceptions. The general explanation for this pattern is that haplotype evolution has been relatively independent of selection, heterogeneity of mutation rates, or major population perturbations during their collective coalescent history. The exceptions are significantly negative *Fs*-values for the Rolinda sample of the Fresno kangaroo rat, the Panoche and Carrizo samples of the short-nosed kangaroo rat, both the Tipton and short-nosed samples overall, and the total pool of haplotypes. Because Fu's *Fs*-value is particularly sensitive to demographic perturbations, significant negative values are interpreted to result from either a selective sweep or population expansion. The distribution of all pairwise differences between haplotypes (mismatch distribution—Rogers and Harpending 1992) supports a signature of population expansion. The pattern is distinctly unimodal and a model of spatial expansion cannot be rejected ($P = 0.502$), although a sudden expansion model was rejected ($P = 0.024$). However, both indications of expansion result from the sharing of just a few haplotypes across much of the species range (Fig. 5).

Table 4.—Estimates of molecular diversity for 18 separate population samples of *Dipodomys nitratoides* (mean ± 1 *SD*), pooled for each subspecies, and for the total sample. * unable to compute as only one haplotype in sample; ** unable to compute because each sampled individual had a different haplotype.

Subspecies/sample	*n*	# haplotypes	Haplotype diversity	Nucleotide diversity	Θ	Θk	95% *CI* Θk
Fresno[a]	48	11	0.821 ± 0.032	0.008 ± 0.005	3.402 ± 1.322	4.160	2.057–8.065
Fresno	2	2	1.000 ± 0.500	0.007 ± 0.009	3.000 ± 3.464	—**	—**
Rolinda	12	8	0.894 ± 0.078	0.007 ± 0.004	3.311 ± 1.593	9.317	3.286–27.264
Lemoore	4	2	0.500 ± 0.265	0.002 ± 0.002	1.000 ± 0.991	0.879	0.182–4.268
Tumbleweed	19	2	0.351 ± 0.111	0.003 ± 0.002	1.053 ± 0.816	0.325	0.075–1.302
Jackson Avenue	11	1	—*	—*	—*	—*	—*
Tipton	60	17	0.930 ± 0.013	0.007 ± 0.004	3.625 ± 1.324	7.550	4.197–13.238
Pixley	3	4	0.603 ± 0.131	0.003 ± 0.003	1.410 ± 1.036	1.574	0.499–4.653
Kern fan-1	11	7	0.909 ± 0.066	0.008 ± 0.005	3.255 ± 2.047	7.237	2.471–21.639
Kern fan-2	12	6	0.849 ± 0.074	0.007 ± 0.005	2.955 ± 1.871	4.101	1.439–11.521
Arvin	14	5	0.725 ± 0.104	0.006 ± 0.004	2.593 ± 1.657	2.331	0.807–6.412
Bakersfield	10	5	0.822 ± 0.097	0.007 ± 0.004	2.756 ± 1.800	3.301	1.068–10.048
Short-nosed	110	26	0.912 ± 0.014	0.009 ± 0.005	4.362 ± 1.372	10.443	6.535–16.357
Los Banos-1	26	3	0.542 ± 0.075	0.006 ± 0.004	2.462 ± 1.532	0.645	0.188–1.9915
Los Banos-2	22	4	0.762 ± 0.044	0.008 ± 0.005	3.186 ± 1.910	1.152	0.382–3.182
Panoche	6	6	1.000 ± 0.096	0.006 ± 0.004	2.333 ± 1.704	—**	—**
Coalinga	18	7	0.817 ± 0.070	0.008 ± 0.005	3.183 ± 1.929	3.720	1.472–9.090
McKittrick	4	3	0.833 ± 0.222	0.005 ± 0.004	2.000 ± 1.678	3.766	0.774–18.233
Carrizo	14	9	0.879 ± 0.079	0.006 ± 0.004	2.538 ± 1.629	9.811	3.708–26.597
Elkhorn	12	7	0.894 ± 0.063	0.006 ± 0.004	2.364 ± 1.558	6.155	2.193–17.418
Cuyama	8	3	0.607 ± 0.164	0.005 ± 0.003	1.857 ± 1.350	1.254	0.334–4.465
Total	218	43	0.941 ± 0.006	0.009 ± 0.005	3.629 ± 2.074	14.341	10.951–22.195

[a] Values include those of the three samples of putative Fresno kangaroo rats (Lemoore, Tumbleweed, and Jackson Avenue).

The population genetic estimate Θ (Table 4) falls well below the 95% confidence limits of Θ_k for the total species pool of haplotypes, suggesting an historical reduction in population size. A trend in historical population reduction also is observed in comparisons of these values for pooled samples within the Tipton and short-nosed populations, but no single-population sample exhibits the same pattern. Data for the Fresno kangaroo rat pooled samples, which suggest no population decline, should be viewed with caution as the majority of the recorded haplotype diversity comes from the Rolinda sample, where the historical skin sample sources of DNA are more likely to result in single-base sequencing errors. The three recent sample sites (Lemoore, Tumbleweed, and Jackson Avenue) possessed the lowest sequence diversity of all freshly sampled populations throughout the range of the species.

The estimated demographic history of *Cytb* haplotypes, and thus of the sampled set of populations across all three subspecies of *D. nitratoides*, is illustrated in the Bayesian skyline plot derived from the BEAST analysis (Fig. 6). Temporal patterns were the same for both constant and linear growth models; results of only the latter are shown. Population size remained either constant or slightly declining from the species origin at about 3.25 Ma (95% high-probability density [HPD] 2.03–4.65 Ma), until approximately 750 Ka when the total species population began a rapid decline, one that reached its lowest point about 200–250 Ka, followed by a very precipitous expansion wherein the species reached a substantially higher total number than during its apparently stable, earlier history. More recent bottlenecks, such as those following the post-World War II habitat conversion, will not be evident at the temporal scale of this representation of deeper history.

Discussion

Morphological versus molecular patterns.—Morphological data largely substantiate each of the three subspecies that have been recognized since Grinnell (1920, 1922, 1933) and corroborated, in part, by Hoffmann (1975). The degree of differentiation among these races, however, varies in strength relative to the three data sets (general size, color, and combinatorial craniodental variables) and across the different transects we analyzed. Nevertheless, the Fresno kangaroo rat (*D. n. exilis*) is well separated by step clines in both PCA and canonical variates analysis (CVA) axes from both the short-nosed kangaroo rat (*D. n. brevinasus*) to the west (Northern transect, Figs. 2–4) and the Tipton kangaroo rat (*D. n. nitratoides*) to the south (Eastern transect, Figs. 2–4). Furthermore, the position of these transitional breaks between short-nosed versus Fresno and Fresno versus Tipton occurs at the respective historically drawn boundaries between both pairs (see Fig. 1). Mid-latitude samples of the short-nosed and northern samples of the Tipton kangaroo rat (the Central transect, Figs. 2–4) also separate by a sharp step in PCA and CVA clines at approximately their putative boundary of Tulare Lake. Importantly, the strength of morphological separation of Fresno from both Tipton and short-nosed kangaroo rats should be sufficient to allocate preserved samples of questionable subspecies attribution (such as the Lemoore, Tumbleweed, or Jackson Avenue samples examined herein), should those become available.

Differences between short-nosed and Tipton kangaroo rats begin to break down across the southern end of the San Joaquin Valley south of the Buena Vista and Kern lake basins. Here, suitable habitat was relatively continuous across the lower bajada of the San Emigdio Mountains, part of the western

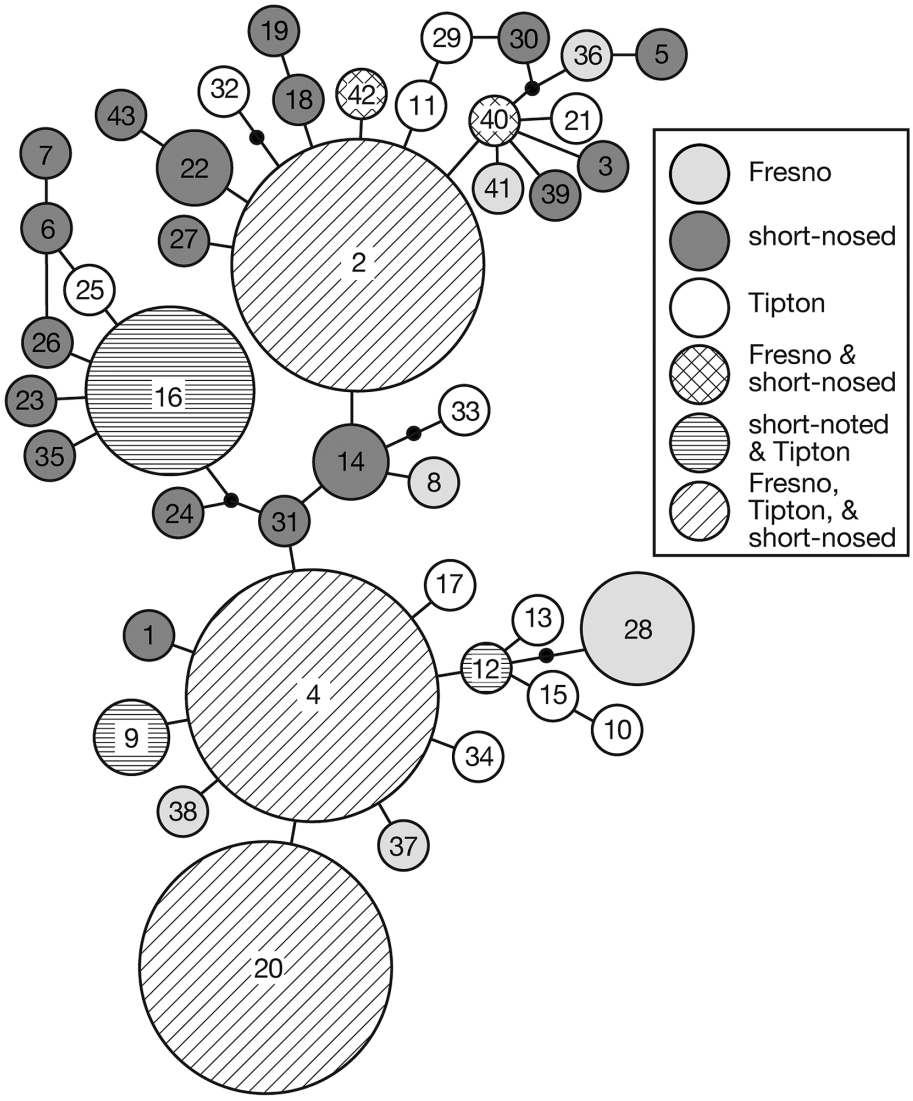

Fig. 5.—Parsimony network of 43 unique mtDNA haplotypes within the sample of 218 specimens of *Dipodomys nitratoides* pattern-coded by distribution within and between subspecies. Lines connecting haplotypes represent single-base substitutions; small black dots represent intermediate haplotypes that were not recovered within the sample; and the size of each circle is proportional to the number of individuals in which the particular haplotype was found.

extension of the Transverse Ranges, presumably providing the opportunity for populations of the two subspecies to interact (Fig. 1). While there is a step cline in both color and combinatorial craniodental variables, but not in size, across this region (Southern transect, Figs. 3 and 4), the positions of the color and craniodental breaks do not coincide with the historically defined allocation of populations to subspecies. We suggest that these discordances simply result from increased gene flow across the region due to lack of substantive barriers.

There is, however, little correspondence between the patterns of morphological differentiation across geography, both within and among the subspecies, and the expression of molecular diversity as measured by mtDNA sequences. Haplotype position within the parsimony network is not partitioned by subspecies (Fig. 5) and the most common and widespread haplotypes are found in one or more population samples of all three, or at least two, of the putative subspecies. AMOVAs indicate little, if any, "subspecies" effect, with only 2% of the total haplotype variation attributed to differences among the pooled samples of each taxon. Even within subspecies, variation is limited, as only 27% is apportioned among samples within taxa. More than 71% of the total variation is found at the individual population level. The same pattern is found when AMOVAs are confined to geographic groups within individual subspecies (data not given). For example, an AMOVA based on grouping short-nosed samples into northern (Los Banos-1, Los Banos-2, Panoche, and Coalinga) versus southern (McKittrick, Elkhorn, Carrizo, and Cuyama) samples partitions only 8.6% of haplotype variation to the geographic group level. Again, most (> 76%) of the variation is found within the local populations. While there is limited clade structure within the parsimony network, the distribution of haplotype diversity is independent of both subspecies boundaries and geography overall (i.e., there is no pattern of isolation by distance).

The lack of concordance between geographic trends in morphology and molecules at the infraspecific level should not be unexpected for any taxon, because the former is usually viewed as reflecting geographic responses to differential selection while the latter reflects lineage history, including temporal depth, of the gene examined as well as population genetic processes of mutation, gene flow, and drift. In part, this difference is why Hennig (1966) explicitly declined to include infraspecific taxonomy and its recognition within his phylogenetic systematics framework. In this species, as well as for most others, a suite of morphological attributes defined subspecies. Our reanalyses of characters employed by Merriam (1894) and Grinnell (1920, 1922), but with a greater number of samples of larger sizes, corroborate their initial arguments when they diagnosed each of the three subspecies of *D. nitratoides*. The lack of a molecular signature concordant with these morphological trends neither negates those patterns nor the taxon definitions based upon them.

Molecular diversity and population history.—The expectation of a direct link between recent demographic history and genetic variability within surviving populations has become a central concern in conservation biology (Frankham et al. 2002). A large number of empirical studies support this link (e.g., Rubidge et al. 2012). There is no doubt that the San Joaquin kangaroo rat has suffered substantial population loss and fragmentation, especially over the past 25–50 years (Williams and Kilburn 1992; U.S. Fish and Wildlife Service 1998). In the discussion that follows, however, we are cognizant that inferences derived from a single locus, as we describe herein, are inadequate to draw confident conclusions about population history of this, or any other species.

Molecular evidence for either loss of variation or increased substructure in the San Joaquin kangaroo rat is, however, either nonexistent or mixed, at best. For example, comparisons of current sample diversity (Θ) relative to estimated effective population size over historic time (Θ_k; Table 4) support a historical reduction in population size and the Bayesian skyline analysis (Fig. 6) clearly identifies a deep bottlenecked trough in the earlier demographic history of the species. Alternatively, neither Tajima's *D* nor Fu's *Fs* indicate historical population perturbations and the mismatch distribution is both unimodal and cannot be distinguished from one generated by spatial expansion. These apparent contradictions likely result from each method providing different insights into, and thus being strongly influenced by, different time slices in the overall population history of the species.

The lack of expected impact of severe population reduction on levels of molecular diversity is not unique to the San Joaquin kangaroo rat. Studies of two other California endemic species, both equally or more greatly impacted by habitat loss and fragmentation than the San Joaquin kangaroo rat, offer comparative, and similar, histories. The Stephens kangaroo rat (*D. stephensi*) inhabits a very small range, estimated at only 40 × 70 km, in one of the most heavily modified regions of southern California, yet Metcalf et al. (2001) found no evidence for reduced levels of haplotype diversity within populations or

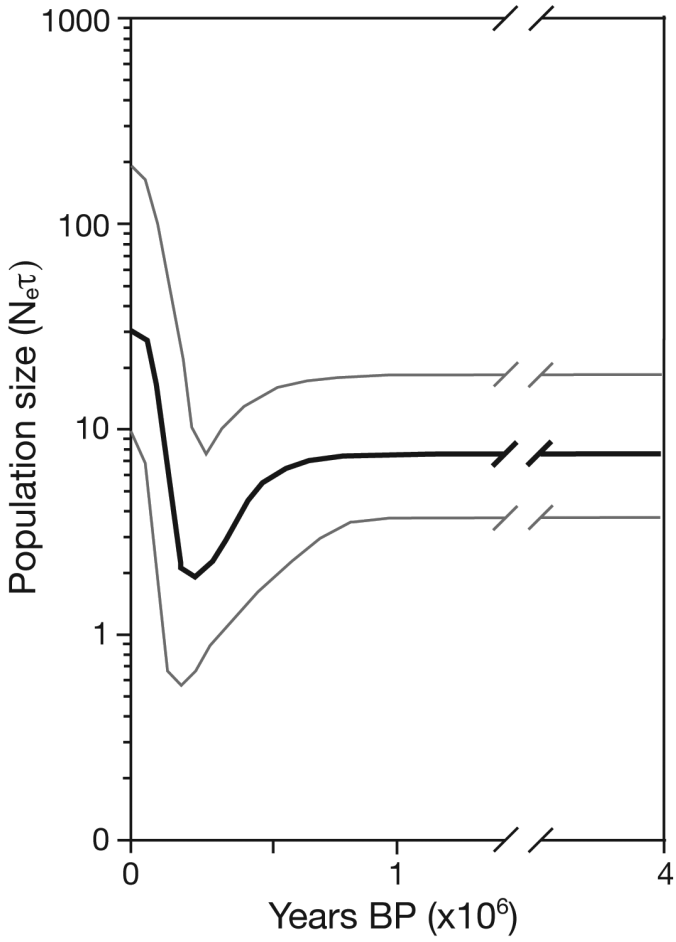

Fig. 6.—Bayesian skyline plot derived from cytochrome *b* sequences for members of the *Dipodomys merriami* clade (*D. phillipsi*, *D. merriami*, and the 43 haplotypes of *D. nitratoides*). The x-axis of the plot is millions of years before the present (BP), using the estimated time to most recent common ancestor (TMRA) for the split between *D. merriami* and *D. nitratoides* of 3.25 ± 0.5 Ma (BEAST analysis of Dipodomyinae and *Dipodomys* using clade dates from Hafner et al. 2007); the y-axis is equal to the product of the effective population size (N_e) and the generation time in years (τ). The thick black line is the median estimate; the thin lines are the 95% high-probability density (HPD) limits. The plot is based on a model in which the population grows or declines linearly between change-points.

haplotype loss in comparison to data from the closely related, more broadly distributed, and nonimpacted Panamint kangaroo rat, *D. panamintinus* (Thomas et al. 1990). Similarly, the giant kangaroo rat (*D. ingens*), which currently exists in only about 2% of its original range on the western side of the San Joaquin Valley, also contains substantial levels of mtDNA haplotype (Good et al. 1997) and microsatellite diversity (Loew et al. 2005). This species' historic range was nearly identical to that of the short-nosed kangaroo rat. Furthermore, while the endangered Morro Bay kangaroo rat, *D. heermanni morroensis*, does have low levels of mtDNA diversity, this was apparently an attribute of its populations well before severe range retraction rather than a result of that bottleneck (Matocq and Villablanca 2001).

Table 5.—Estimates of Tajima's *D* and Fu's *Fs* (with probability of significant deviation from neutral expectations, based on 10,000 random permutations), for each sample, subspecies, and total haplotype pool. Significant values are indicated in bold.

Subspecies/sample	Tajima's *D*	Fu's *Fs*
D. n. exilis[a]	−0.164 ($P = 0.495$)	−0.805 ($P = 0.403$)
Fresno	0.000 ($P = 0.999$)	1.099 ($P = 0.432$)
Rolinda	−0.707 ($P = 0.263$)	**−2.864** ($P = 0.031$)
Lemoore	−0.710 ($P = 0.281$)	1.099 ($P = 0.628$)
Tumbleweed	0.616 ($P = 0.757$)	3.031 ($P = 0.910$)
Jackson Avenue	—	—
D. n. nitratoides	−0.513 ($P = 0.353$)	**−5.198** ($P = 0.029$)
Pixley	−0.999 ($P = 0.186$)	0.240 ($P = 0.538$)
Kern fan-1	0.793 ($P = 0.801$)	−1.447 ($P = 0.170$)
Kern fan-2	1.075 ($P = 0.872$)	−0.333 ($P = 0.417$)
Arvin	0.117 ($P = 0.591$)	0.735 ($P = 0.669$)
Bakersfield	−0.110 ($P = 0.477$)	0.127 ($P = 0.519$)
D. n. brevinasus	−0.504 ($P = 0.354$)	**−9.516** ($P = 0.006$)
Los Banos-1	1.674 ($P = 0.955$)	4.577 ($P = 0.972$)
Los Banos-2	1.483 ($P = 0.940$)	3.669 ($P = 0.945$)
Panoche	0.367 ($P = 0.658$)	**−3.927** ($P = 0.003$)
Coalinga	−0.017 ($P = 0.548$)	−0.063 ($P = 0.507$)
McKittrick	−0.780 ($P = 0.204$)	−0.134 ($P = 0.342$)
Carrizo	−0.750 ($P = 0.248$)	**−3.803** ($P = 0.011$)
Elkhorn	0.077 ($P = 0.564$)	−2.045 ($P = 0.076$)
Cuyama	0.889 ($P = 0.824$)	1.412 ($P = 0.784$)
Total	−1.110 ($P = 0.116$)	**−24.589** ($P = 0.000$)

[a] Includes the putative Fresno kangaroo rats from Lemoore, Tumbleweed, and Jackson Avenue.

With the exceptions of the three samples from Naval Air Station Lemoore (Lemoore, Tumbleweed, and Jackson Avenue; Table 4), for which estimated haplotype diversity is quite low (only two haplotypes among the 19 individuals from Tumbleweed and the 11 specimens of the Jackson Avenue sample all possess the same haplotype), other extant populations of the San Joaquin kangaroo rat contain reasonable levels of molecular diversity (the mean haplotype diversity for those samples of 10 or more individuals is 0.809; Table 4). To the degree to which this single mtDNA marker is a proxy for diversity across nuclear gene loci, this species as a whole, its constituent subspecies, and most local populations have apparently retained adequate genetic variation suitable for an adaptive response to changing environmental conditions. Furthermore, most local populations must have maintained reasonable effective sizes despite the reduction and fragmentation experienced over most of the species' range. Nevertheless, one should not take what appears to be a reasonable pool of current standing molecular variation as an indicator of the long-term genetic health of *D. nitratoides*. If current fragmentation persists, or certainly if it is exacerbated, erosion of this standing variation is inevitable.

Phyletic history and biogeography.—Three attributes of the molecular data we present here that stand out are 1) the low level of molecular divergence among haplotypes (~1%), 2) the very shallow gene tree built almost exclusively from single-mutation steps (Fig. 4), and 3) the lack of any significant geographic structure. Consequently, the coalescent time for these haplotypes must be quite recent, at least with respect to the age of the species itself. The BEAST analysis using date-calibrated nodes from Hafner et al. (2007) estimated the split between *D. merriami* and *D. nitratoides* at 3.25 Ma, with achievement of reciprocal monophyly of their respective mtDNA necessarily more recently.

The coalescent time, or time to most recent common ancestor (TMRA), of all recovered haplotypes of *D. nitratoides* can be estimated in numbers of generations (t) by $\tau/2\mu$, where μ is the mutation rate and τ is the age of expansion. Empirical estimates of τ derived from a model of spatial expansion (which cannot be rejected as underlying the pairwise mismatch distribution; Fig. 5) are a mean = 3.886 with 95% confidence limits of 0.999 and 5.263, or about 149,500 generations (range 38,400–202,400). Limited data on population demography for the San Joaquin kangaroo rat suggest an annual turnover and maximum longevity of 3–5 years (U.S. Fish and Wildlife Service 1998), with a generation time less than the 1.7 years estimated for the larger bodied banner-tailed kangaroo rat, *D. spectabilis* (Busch et al. 2007). A conservative generation time of 1.5 years yields a coalescent time for the haplotype network of 224,200 years ago (range 57,600–303,630), or approximately 10% of the time since the origin of the species and perhaps 20% since *D. nitratoides* and *D. merriami* achieved reciprocal monophyly for their respective *Cytb* genes (Avise et al. 1987). Why, then, is the depth of the gene tree so shallow with respect to the depth of the species' tree?

Dipodomys nitratoides most likely split as a peripheral isolate from the more widespread *D. merriami*, either by dispersal through the low pass where the Tehachapi Mountains meet the Transverse Ranges, the ranges that today separate the two species, or as a result of vicariance if these ranges uplifted coincidentally. The substantial temporal difference between the time of origin and haplotype coalescence, however, suggests that *D. nitratoides* was either confined to a small portion of the San Joaquin Valley for an extended period of time in its early history or retracted from a wider range before it expanded again to its present distributional limits. Much of the valley floor itself would have been uninhabitable by kangaroo rats until recently because of riparian forests and lakes and associated wetlands. The limited understanding of the history of the Tulare and Buena Vista lake basins is generally consistent with the estimated time since the most recent ancestor of the recorded haplotypes. The uplift of the Coastal Ranges (to the west of these basins) was complete by about 615,000 years ago, closing the southern outlet of the Tulare Basin to the Pacific Ocean and forming a rain shadow over the valley floor. The large lake that filled the basin prior to this time gradually diminished, and the increasing aridity from the newly formed rain shadow produced habitat suitable for expanding kangaroo rat populations by about 200,000 years ago (Davis and Cophen 1989). This history is quite concordant with the estimated TMRA for the haplotype network, despite the large uncertainties in those estimates resulting from the small fragment of DNA sequenced. This same temporal history is argued to have resulted in the measured haplotype diversity and structure for the partially overlapping giant kangaroo rat (Good et al. 1997).

Management implications.—The trend of loss of habitat for *D. nitratoides* that Grinnell (1920) recognized early in the San Joaquin Valley has only been exacerbated in the post-World War II expansion of water projects and agribusiness (see Kelly et al. 2005). The U.S. Fish and Wildlife Service (1998) estimated that viable populations currently occupy only about 2% of the pre-1850s species' range. Today, of the three recognized subspecies, the Fresno kangaroo rat is either extinct or only survives on fewer than six tiny, isolated parcels at the northern edge of historical Tulare Lake (completely drained by the 1950s—Arax 2019), while the Tipton and short-nosed kangaroo rats occupy greatly reduced and very fragmented ranges. At the molecular level, *D. nitratoides* and its component extant populations appear to have retained significant genetic diversity despite a recent history of severe and rapid population retraction. Fortunately, the morphological differences we have identified between subspecies suggest that allocation of newly discovered populations to subspecies might be feasible from a few preserved specimens, certainly more so than obtaining mtDNA sequences from tissue biopsies alone. While a robust measurement of nuclear gene variability would be of value, management protocols should focus on preserving habitat and demographic attributes of local populations rather than captive or other breeding protocols designed primarily to maintain a pool of genetic diversity. The historical demography of extant populations has apparently been adequate for this important purpose.

Given that all three subspecies of San Joaquin kangaroo rat remain either listed as Endangered or as California Species of Special Concern, we follow the lead of Cypher et al. (2017) in supporting range-wide management actions for the species.

1. There are significant information gaps in our understanding of the current distribution of the species. This is in part due to the fact that many parcels with potential habitat are privately owned and have limited or no access for surveys. Whenever such parcels become accessible, surveys should be a high priority. We encourage the U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife to develop incentive programs to enhance cooperation by private landowners on surveying for San Joaquin kangaroo rats. Particularly, high priority should be given to locating extant populations of *D. n. exilis*, as no individuals have been seen since 1992. Furthermore, the current population-level status of *D. n. brevinasus* shoulwd be assessed to determine whether additional protections are warranted.
2. Locations on the San Joaquin Valley floor where *D. nitratoides* have been confirmed, but that are not permanently protected, should be a high priority for habitat conservation. This includes populations of *D. n. brevinasus*. Locations that have high-quality habitat also should be a priority for conservation even if no survey data are currently available for those sites. An ideal conservation strategy would be one in which a network of reserves with connectivity is established for each subspecies and across the entire species' range. Unfortunately, the Fresno kangaroo rat is most likely extinct. Furthermore, the agroecosystem that comprises most of the San Joaquin Valley will make connectivity within and among the remaining populations of both short-nosed and Tipton kangaroo rats impossible without large-scale reclamation and restoration to native habitats.
3. Over the past few decades, it has become apparent that *D. nitratoides* populations are threatened by habitat degradation as well as by habitat loss and fragmentation. Degradation is usually due to invasion by non-native grasses. These grasses must be managed to maintain habitat suitability for kangaroo rats. The most cost-effective and efficient means to do this is through the use of livestock grazing. San Joaquin kangaroo rats were extirpated at the Lemoore Naval Air Station and Allensworth Ecological Reserve (ESRP) after grazing was terminated and the density of non-native grasses increased. In contrast, in a long-term study at the Lokern Natural Area in Kern County, short-nosed kangaroo rats increased significantly (73%) on cattle-grazed plots compared to ungrazed plots (Germano et al. 2012).
4. Through a scientifically rigorous habitat restoration program, formerly cultivated lands—retired agricultural lands—could play a significant role in *D. nitratoides* conservation. Such restored habitat may help offset ongoing habitat loss (see also point 2, above).
5. Further research needs to be conducted on more effective methods for using translocation as a conservation strategy for *D. nitratoides*. This important conservation technique has the potential to help establish populations in vacant, isolated parcels or in restored habitat areas (see point 4), but research is urgently needed to increase the probability of translocation success rates.

These actions in combination may help to stem the range-wide decline of this species and its habitat that has been ongoing for over a century (Kelly et al. 2005).

Acknowledgments

R. Robertson gathered the initial set of sequences as part of an undergraduate honors thesis in 1993. A. Selas performed the more recent laboratory analyses, including those involving museum specimens. Robertson's and subsequent laboratory work through 1996 was undertaken under contract with the U.S. Bureau of Reclamation in conjunction with the Endangered Species Recovery Program (ESRP) via funds from Central Valley Project Improvement Act and the Central Valley Conservation Program administered by the U.S. Bureau of Reclamation and the U.S. Fish and Wildlife Service (USFWS). These agencies and the California Department of Fish and Wildlife (CDFW) financially supported additional geographic surveys for Fresno kangaroo rats by ESRP personnel, whom we thank en masse for collecting the ear biopsies from trapped animals before their release. Trapping and biopsy of tissue from live animals was authorized under scientific collecting

permits and memoranda of understanding issued by CDFW to DFW and PAK, and Endangered Species permits issued by the USFWS. W. Hoffmann graciously provided a copy of his master's thesis on the Fresno kangaroo rat to JLP. Finally, we thank D. Wilson and R. Fisher (USNM) and D. Grubbs and C. Kronberg (CSUF) for access to specimens in their respective collections, and J. Esselstyn and two anonymous reviewers whose critical eyes greatly improved the manuscript.

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Maps of specimen localities, place names, and historical vegetation communities of the San Joaquin Valley and adjacent areas (Fig. 1) and the present-day modified landscapes of the same region (Fig. 2).

Supplementary Data SD2.—Geographic groups used in craniodental comparisons, including their sample sizes, individual localities, geographic coordinates, and specimen numbers.

Supplementary Data SD3.—Geographic groups used in colorimetric comparisons, including their sample sizes, individual localities, geographic coordinates, and specimen numbers.

Supplementary Data SD4.—Geographic groups used in molecular comparisons, including their sample sizes, individual localities, geographic coordinates, and specimen numbers.

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Submitted 25 April 2019. Accepted 16 July 2019.

Associate Editor was Jacob Esselstyn.